This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

Document AM3
Appl. No. 09/641,528

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/025, 16/08, C12N 15/37, A61K 39/12, G01N 33/569

A1

(11) International Publication Number: **WO 95/31476**

(43) International Publication Date: 23 November 1995 (23.11.95)

(21) International Application Number: PCT/AU95/00292

(22) International Filing Date: 17 May 1995 (17.05.95)

(30) Priority Data:
PM 5667 17 May 1994 (17.05.94) AU

(71) Applicant *(for all designated States except US)*: THE UNIVERSITY OF QUEENSLAND [AU/AU]; St. Lucia, QLD 4072 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: FRAZER, Ian [AU/AU]; 110 Highland Terrace, St. Lucia, QLD 4067 (AU). ZHOU, Jian [AU/US]; Loyola University, 2160 South First Avenue, Maywood, IL 60153 (US).

(74) Agent: KELLY, Robin, Thomas; Fisher & Kelly, Level 1, 349 Coronation Drive, P.O. Box 1477, Milton, QLD 4064 (AU).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

(54) Title: RECOMBINANT PAPILLOMA VIRUS L1

(57) Abstract

This invention relates to a recombinant papilloma virus L1 protein which can elicit an immune response which recognises papilloma virus VLP including L1 protein and can form extracellularly a multimeric structure or VLP wherein the multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins. This invention also includes the use of the recombinant papilloma virus L1 protein to detect the presence of papilloma virus and can form the basis of a vaccine for prophylactic and therapeutic use.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

TITLE

RECOMBINANT PAPILLOMA VIRUS L1

FIELD OF THE INVENTION

THIS INVENTION relates to the L1 protein papilloma viruses. In particular, the invention relates to recombinant papilloma virus L1 protein and its use for detecting and treating papilloma virus infections.

BACKGROUND OF THE INVENTION

Papilloma viruses infect a range of hosts including man, cattle, sheep, dogs and cats. For a more complete listing, see "Papilloma Virus Infections in Animals" by J. P. Sundberg which is described in Papilloma Viruses and Human Diseases, edited by K. Syrjanen, L. Gissman and L. G. Koss, Springer Verlag, 1987.

Human papilloma viruses induce benign hyperproliferative lesions of the cutaneous and mucosal epithelia. Of the 70 different virus types which infect humans, more than 20 are associated with anogenital lesions (de Villiers, 1989, J. Virol. **63** 4898-4903). Papilloma viruses have also been associated with various forms of cancers. Human papilloma virus types 16 and 18 have been associated with a number of cervical intra-epithelial neoplasias and carcinomas of the cervix (Lancaster *et al.*, 1987, Cancer Metast. Rev. **6** 6653-6664 and Pfister, 1987, Adv. Cancer Res. **48** 113-147).

Papilloma viruses are small DNA viruses encoding up to eight early and two late genes. The late genes L1 and L2 code for structural proteins which assemble into a capsid within the cell (Galloway *et al.*, 1989, Adv. Virus Res. **37** 125-171). A single virus capsid is a T=7d icosahedron composed of 360 pentameric capsomers, each of which contains five molecules of the major capsid protein L1 (Baker *et al.*, 1991, Biophys. J. **60** 1445-1456 and Finch *et al.*, 1965, J. Mol. Bio. **13** 1-12). The minor capsid protein L2 is present at approximately one-tenth the abundance of L1 (Doorbar *et al.*, 1987, J. Virol. **61** 2793-2799).

Propagation of human papilloma viruses *in vitro* has not been achieved (Taichman *et al.*, 1984, J. Invest. Dermatol. **83** 25) and

only small amounts of HPV proteins have been isolated from infected tissues (Androphy *et al.*, 1987, Embo J. **6** 1989; Banks *et al.*, 1987, J. Gen. Virol. **68** 1351; Firzlaff *et al.*, 1988, Virology **164** 467; Oltersdorf *et al.*, 1987, J. Gen. Virol. **68** 2933; Schneider-Gadicke *et al.*, 1988, Cancer Res. **48** 2969; Seedorf *et al.*, Embo J. **6** 139 and Smotkin *et al.*, 1986, PNAS **83** 4680). However, the gene coding for L1 protein has been cloned and expressed in a eukaryotic expression system using recombinant vaccinia virus (Browne *et al.*, 1988, J. Gen. Virol. **69** 1263-1273; Zhou *et al.*, 1990, J. Gen. Virol. **71** 2185-2190 and Zhou *et al.*, 1991, Virology **185** 251-257), in a baculovirus expression system (Park *et al.*, 1993, J. Virol. Meth. **45** 303-318) and in a bacterial expression system (Steubne *et al.*, 1989, J. Gen. Virol. **70** 543-555).

As L1 protein is the major capsid protein, it has been used as the basis for the development of vaccines for protection against papilloma virus infection. Zhou *et al.* immunized mice with synthetic HPV16 virus-like particles (VLPs) using a vaccinia virus doubly recombinant for the L1 and L2 proteins of HPV16. The murine anti-VLP anti-sera recognised HPV16 capsids by ELISA and baculovirus recombinant HPV16L1 and L2 protein on immunoblot. The murine anti-VLP anti-sera, however, failed to recognise two peptides that were recognised by anti-HPV16L1 monoclonal antibodies raised against a recombinant L1 fusion protein (Zhou *et al.*, 1992, Virology **189** 592-599). These researchers concluded that the immunoreactive epitopes of HPV16 defined using virus-like particles differ significantly from those defined using recombinant HPV16L1 fusion proteins.

To overcome problems of presentation, vaccines were developed using virus-like particles. VLPs were formed intracellularly from recombinant L1 or L1 and L2 proteins encoded by recombinant vaccinia virus (Zhou *et al.*, 1991, Virology **185** 251-257; Zhou *et al.*, 1991, Virology **181** 203-210 and International Patent Specification WO93/02184). These vaccines using synthetic virus-like particles have a number of disadvantages. Firstly, the recombinant L1 or L1 and L2 genes are

expressed from a vaccinia virus vector which may not be suitable for the production of a vaccine. Secondly, the virus-like particles are produced intracellularly which is a rate limiting step. Thirdly, the virus-like particles may incorporate cellular DNA because they are produced intracellularly and virus-like particles incorporating DNA are not suitable for use in vaccines. Fourthly, virus-like particles may only be partially purified because of the need to retain their integrity and hence correct epitope presentation. Consequently, other proteins or matter associated with the virus-like particles may contaminate a vaccine preparation. Fifthly, the process of producing a vaccine in commercial amounts with virus-like particles from recombinant vaccinia viruses is comparatively expensive.

Similar disadvantages apply to the use of the virus-like particles produced from recombinant vaccinia viruses for the detection of antibodies in the sera of patients.

SUMMARY OF THE INVENTION

The present invention results from the surprising discovery that a recombinant papilloma virus L1 protein can form multimeric structures extracellularly and elicit an immune response that recognises native papilloma virus capsids.

Thus it is an object of the present invention to provide a recombinant papilloma virus L1 protein suitable for use in prophylactic and therapeutic vaccines and detection assays that overcomes one or more of the aforementioned problems.

In one aspect, the invention provides a recombinant papilloma virus L1 protein which is characterised by its ability to elicit an immune response which recognises papilloma virus VLP including L1 protein and to form extracellularly a multimeric structure wherein said multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins.

The recombinant papilloma virus L1 protein may be a fusion protein. A suitable fusion protein includes $(His)_6$--papilloma virus L1 protein. A suitable fusion protein may be HPV6b L1 HEXAHIS protein.

Alternatively, another papilloma virus L1 protein of a different type may be used. The recombinant papilloma virus L1 protein may be an entire or partial amino acid sequence of a papilloma virus L1 protein. The recombinant papilloma virus L1 protein may be an amino acid sequence coding for one or more epitopes that elicit an immune response which recognises papilloma virus VLP including L1 protein. A suitable recombinant papilloma virus L1 protein is given in FIG. 1. This recombinant papilloma virus L1 protein is given by way of example only.

The immune response elicited by the recombinant papilloma virus L1 protein may be an antibody response, or an antibody response along with a cell mediated response. The elicited response may recognise recombinant and/or native papilloma virus VLP L1 protein. The antibody response is where antibodies are raised against the recombinant papilloma virus L1 protein and these antibodies recognise papilloma virus VLP L1 protein. The cell mediated and humoral response may include T cells, large granular lymphocytes, mononuclear phagocytes, neutrophils, eosinophils, basophils, mast cells, various tissue cells, platelets, complement, inflammatory mediators and cytokines including interferons, interleukins, colony stimulating factor, tumor necrosis factor and transforming growth factor B. The cell mediated and humoral response may result from being primed and challenged with recombinant papilloma virus L1 protein. A suitable cell mediated and humoral response is the development of delayed type hypersensitivity.

The multimeric structure may be any size but preferably it is a pentameric structure. A multimeric structure may be a VLP. VLP includes papilloma virus virions and recombinant VLP. The multimeric structures are formed extracellularly and preferably after the recombinant papilloma virus L1 protein has been substantially purified. The multimeric structures may self-assemble in suitable buffers. The invention also provides a multimeric structure formed extracellularly and comprising a plurality of recombinant papilloma virus L1 proteins. A multimeric structure as defined above may be a pentameric VLP.

The ability of a papilloma virus L1 protein to elicit an immune response which recognises papilloma virus VLP including L1 protein requires correct presentation of appropriate epitopes. Recombinant papilloma virus L1 proteins that do not form VLP do not induce an immune response which recognises papilloma virus VLPs including L1 protein. Recombinant GST papilloma virus L1 protein, recombinant MS2 papilloma virus L1 protein and denatured papilloma virus L1 protein do not elicit an immune response which recognises papilloma virus VLP including L1 protein. All VLPs to date have been produced intracellularly with the expression of papilloma virus L1 or L1 and L2 genes. The recombinant papilloma virus L1 protein of the present invention correctly presents one or more epitopes to elicit an immune response which recognises papilloma virus VLP including L1 protein. The recombinant papilloma virus L1 protein of the present invention can form the multimeric structures or VLPs extracellularly. It is believed that the multimeric structures of VLPs formed from the recombinant papilloma virus L1 protein correctly presents one or more epitopes to elicit an immune response which recognises papilloma virus VLPs including L1 protein. Therefore, the invention provides a recombinant papilloma virus L1 protein which can form extracellularly a multimeric structure or VLP which can elicit an immune response which recognises papilloma virus VLP including L1 protein wherein said multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins.

The fact that the multimeric structures or VLPs can be formed extracellularly overcomes a number of problems associated with intracellular VLP formation. These problems include low VLP levels, the possibility of incorporating DNA in the VLP and the possible loss of integrity of the VLP with purification.

A second aspect of the invention is a recombinant DNA molecule which encodes the recombinant papilloma virus L1 protein according to the first aspect of the invention. The recombinant DNA molecule may encode a part of said recombinant papilloma virus L1

protein that includes the epitopes that elicit an immune response which recognises papilloma virus VLP including L1 proteins. Alternatively, the recombinant DNA molecule may be a synonymous DNA sequence that codes for said recombinant papilloma virus L1 protein or said part of said recombinant papilloma virus L1 protein. The recombinant DNA molecule may encode a sequence that can hybridise under standard conditions to a sequence encoding said recombinant papilloma virus L1 protein or said part of said recombinant papilloma virus L1 protein. A suitable recombinant DNA molecule is provided in FIG. 1.

A third aspect of the invention is a method for the preparation of the recombinant papilloma virus L1 protein according to the first aspect of the invention including the steps of:-

(I) expressing a recombinant DNA molecule which encodes the recombinant papilloma virus L1 protein to form said recombinant papilloma virus L1 protein; and

(II) purifying the recombinant papilloma virus L1 protein.

The recombinant DNA molecule may be constructed from a suitable source of papilloma virus DNA such as a human papilloma virus or a bovine papilloma virus using standard cloning and/or PCR techniques. The recombinant DNA molecule may also include an expression vector. The expression vector may be a plasmid, cosmid, phagemid or a virus. A suitable expression vector encodes (in the following order) an ATG site, $(His)_6$ peptide, , and then a cloning site wherein papilloma virus L1 protein DNA sequence may be inserted in the correct reading frame so that a fusion protein of $(His)_6$-L1 protein results from translation. A preferable expression vector is any one of plasmids pTrcHisA, pTrcHisB and pTrcHisC.

The said recombinant papilloma virus L1 protein may be produced by expression of said recombinant DNA molecule. Expression may occur *in vivo* or *in vitro*.

Suitable *in vitro* expression systems include bacterial

expression systems including *E. coli* and any suitable aforementioned expression vector, or eukaryotic expression systems including insect cells such as *Spodoptera frugiperda*, CHO cells, chicken embryo fibroblasts, BHK cells, human SW13 cells, drosophila cells, yeast cells, mosquito cells derived from *Aedes albopictus* or monkey epithelial cells and any suitable expression vector including yeast plasmids, baculovirus, vaccinia virus, Sindbus virus, SV40, Sendai virus, adenovirus, retrovirus or pox viruses. The preferred expression system is a bacterial expression system with *E. coli* and plasmid pTrcHisB. Introduction of the recombinant DNA molecule into a suitable host can be achieved by any suitable method including transfection and transformation. A preferable recombinant DNA molecule is the complete DNA sequence of HPV6b L1 protein inserted into pTrcHisB in a correct reading frame orientation to form pTrc6bL1. The recombinant DNA molecule pTrc6bL1 is preferably transformed into *E. coli* strain DH5.

Following expression, the expression system may be disrupted. Where the expression system is a cell system, the cell may be lysed with suitable techniques and agents such as sonication in a buffer containing guanidinium hydrochloride. The recombinant papilloma virus L1 protein may be partially or completely purified. Purification may be achieved by using any one or more suitable chromatographic procedures. Where the recombinant papilloma virus L1 protein includes a sequence of approximately six histidines, the recombinant papilloma virus L1 protein may be purified using a step of affinity chromatography with a nickel column. Additional purification steps may include preparative gel electrophoresis.

In a fourth aspect, the invention provides a method for detecting the presence of papilloma virus.

The method may detect the presence of papilloma virus L1 protein in a sample using antibody raised against said papilloma virus L1 protein. The method may employ ELISA, RIA or other immunoassay techniques. The method may include the steps of:-

(1) coating the wells of a microtitre plate with a sample which putatively contains papilloma virus L1 protein;

(2) adding antisera raised against the recombinant papilloma virus L1 protein to form a papilloma virus L1 protein-antibody complex; and

(3) detecting the presence of the papilloma virus L1 protein-antibody complex with a detection agent.

With respect to step (1), the wells of the microtitre plate may be initially coated with antisera raised against the recombinant papilloma virus L1 protein prior to the addition of the sample. The detection agent may be an antibody or other suitable ligand conjugated with a suitable label. A suitable label may include any suitable enzyme label such as horseradish peroxidase, a radioactive isotope or a fluorometric molecule.

Alternatively, the method may detect the presence of antibodies specific for papilloma virus L1 proteins in a sample using said recombinant papilloma virus L1 protein.

The method may employ ELISA, RIA or other immunoassay techniques. The method may include the steps of:-

(i) coating the wells of a microtitre plate with the recombinant papilloma virus L1 protein;

(ii) adding the sample which putatively contains antibody specific for papilloma virus L1 protein to form a recombinant papilloma virus L1 protein-antibody complex; and

(iii) detecting the presence of recombinant papilloma virus L1-antibody complex with a detection agent.

The invention also provides a kit for detecting the presence of papilloma virus L1 protein in a sample and includes antibody raised against said recombinant papilloma virus L1 protein.

Further, the invention provides for a kit for detecting the presence of antibody specific for papilloma virus L1 protein in a sample and includes said recombinant papilloma virus L1 protein.

In a fifth aspect, the invention provides for a prophylactic and therapeutic vaccine including said recombinant papilloma virus L1 protein. The vaccine may include a suitable adjuvant such as ISCOMS, alum, Freunds Incomplete adjuvant, Freunds Complete adjuvant, Quil A, other saponins, Aluminium hydroxide algammulin, and pertussigen. Alternatively, the vaccine may not include adjuvant where the recombinant papilloma virus L1 protein is immunogenic without adjuvant.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the DNA nucleotide sequence and amino acid sequence of HPV6bL1HEXAHIS protein; and

FIG. 2 is an electron micrograph of pentameric structures of HPV6bL1 HEXAHIS protein aggregates.

Reference may now be made to various preferred embodiments of the invention. In these preferred embodiments, it should be noted that the references to specific papilloma viruses, vaccines and constructs of recombinant DNA molecules are given by way of example only.

EXPERIMENTAL

EXAMPLE 1: Production of HPV6b L1 HEXAHIS protein

Construction of pTRC6bL1

The L1 open reading frame of HPV6b was cloned from a clinical isolate by polymerase chain reaction using as primers:

GCGGATCCAGATGTGGCGGCCTAGCGACAGCACAGTATATG

and

CGCCCGGGTTACCTTTTAGTTTTGGCCTCGCTTACGTTTTAGG.

The resulting 1.5kb PCR product was cleaved with *Bam*H1 and *Sma*1 and cloned into a *Bam*H1/klenow blunted *Eco* R1 site created within the plasmid pTRCHIS B (Invitrogen). The resultant L1 recombinant plasmid was pTRC6bL1 and encodes a protein sequence:

Met.Arg.Gly.Ser.His.His.His.His.His.His.Gly.Met.Ala.Ser.Met.Thr.Gly.Gly.Gln.Gln.Met.Gly.Arg.Asp.Leu.Tyr.Asp.Asp.Asp.Lys.Asp. (HPV6b L1 aas 1-520).

Growth of Bacteria encoding the HPV6b L1 HEXAHIS Protein

10 mls of 2YT broth (16 mg tyrptone, 10 mg yeast, 5 mg NaC1) containing Ampicillin (final concentration 100 µg/ml) was inoculated with 10 µl of one loopful of bacteria (*E. coli* DH5) from glycerol stock. The culture was incubated at 37°C with aeration at 120 rpm for six hours.

200 mls of 2YT broth containing Ampicillin (final concentration 100 µg/ml) was inoculated with the six hour-10 ml culture. The culture was incubated at 37°C with aeration at 120 rpm overnight.

800 mls of 2YT broth containing Ampicillin (final concentration 100 µg/ml) was inoculated with the 200 ml-overnight culture. The culture was incubated at 37°C with aeration at 120 rpm until the absorbance reached between 0.6 - 0.8 O.D. units at 600 nm (usually 2-3 hours). The HPV6b L1 HEXAHIS protein was induced by addition of 0.5 mM IPTG for 4-6 hours.

The bacteria were pelleted by centrifugation (Beckman JA14 rotor centrifuged at 5000 rpm for 10 minutes at 20°C). The pellet was washed in 50 ml of phosphate buffered saline by resuspending the bacterial pellet in a 50 ml centrifuged tube. The washed bacteria were repelleted by centrifugation (Beckman TJ-6 at 3000 rpm for 10 minutes at 20°C). The supernatant was discarded. The pellet was stored at -20°C or -70°C until needed.

Purification of HPV6b L1 HEXAHIS Protein

The bacteria were resuspended and lysed in 50 ml of Guanidinium lysis buffer (6M Guanidinium hydrochloride and 5.8 ml/litre of solution A [177 mM NaH_2PO_4 and 5M NaCl] pH 7.8 using HCl). The suspension was sonicated at 30% output for two minutes. The cell debris was pelleted by centrifugation (Beckman JA21 rotor at 10000 rpm for 30 minutes at 4°C). The supernatant which contains the HPV6b L1 HEXAHIS protein was retained.

The HPV6b L1 HEXAHIS protein was substantially purified by essentially a two step purification procedure.

The supernatant containing the HPV6b L1 HEXAHIS protein

was loaded onto a nickel column (2.6 cm x 6 cm) using a BIORAD ECONO system at 4°C. Before loading the supernatant, the column was washed thoroughly with NA buffer at 1 ml/minute. NA buffer comprises 6M urea, 5.8 mls/litre solution A [177 mM NaH_2PO_4 and 5 M NaCl], 94 mls/litre solution B [200 mM Na_2HPO_4 and 5 M NaCl] at pH 7.8 using HCl before urea was added. The supernatant was loaded onto the Nickel column at one ml/minute. 10 ml fractions were collected in case the column was overloaded and any unbound protein was washed through the column. After the supernatant was loaded, the column was washed with NB buffer at a flow rate of one ml/minute. NB buffer comprises 6 M urea and 100 mls/litre of solution A [177 M NaH_2PO_4 and 5 M NaCl] at pH 4.0 using HCl before urea is added. The column was washed with NB buffer according to the procedure in Table 1 where lowering of the pH gradient removed contaminating proteins. 10 ml fractions of the eluent were collected.

The fractions containing HPV6b L1 HEXAHIS protein were determined by either dot blot, direct ELISA or SDS PAGE. After the fractions were identified, the washing of the column continued with 100% NB buffer until the pH levelled off. The column was then washed with NA buffer. (The column was stored in 20% ethanol.)

The fractions containing HPV6b L1 HEXAHIS protein were pooled and dialysed against five litres of dH_2O or 10 mM Tris HCl pH 7.5 for overnight at 4°C (or two hours at room temperature). The protein was then precipitated with acetone in a 8:2 acetone to sample ratio, for two hours at -70°C or overnight at -20°C. The protein-acetone solution was centrifuged (Beckman TJ-6 at 3000 rpm and at 4°C for 20 minutes). The supernatant was discarded. The pellet was dried under a flow of nitrogen gas for five minutes to remove any remaining acetone.

The pellet was resuspended in 1 ml of ddH_2O and 4-5 mls of 4 x loading buffer [1.0 ml of 0.5M Tris pH 6.8, 0.8 ml of glycerol, 1.6 ml of 10% SDS w/v, 0.1 g of DTT 1% w/v, 0.2 ml of 0.1% w/v bromphenol blue and 4.4 ml of dH_2O]. The resuspended pellet was heated at 65-70°C for

15 minutes to ensure all the protein was dissolved.

The resuspension was loaded onto a BIORAD Prep Cell comprising a 10% separating gel (4.5 cm high by 4 cm diameter) with a 4% stacking gel (4 cm high by 4 cm diameter). The Prep Cell was ran at 12W constant power.

When the dye front of the gel reached 2 cm from the bottom, 10 ml fractions at a 1 ml/minute elution rate were collected. Fractions were tested for HPV6b L1 HEXAHIS protein by either dot blot, direct ELISA or SDS PAGE (with the Phast system). Positive fractions were tested on SDS PAGE and those found to have a single HPV 6bL1HEXAHIS protein band were pooled. The pooled fractions were dialysed against 5 litres of ddH_2O to remove glycine. Dialysis occurred overnight at a temperature of 4°C and using two changes of ddH_2O.

The dialysed HPV6b L1 HEXAHIS protein was precipitated with acetone to remove SDS. A 8:2 acetone to sample ratio was used either for two hours at -70°C or overnight at -20°C. The protein-acetone solution was centrifuged (Beckman TJ-6 at 3000 rpm and at 4°C for 20 minutes). The supernatant was discarded and the pellet was dried under a flow of nitrogen gas for five minutes to remove any remaining acetone.

The protein was then able to be resuspended in a buffer of choice and its concentration determined. This protein was subsequently demonstrated to form capsomers by purifying the HPV6b L1 HEXAHIS protein as described , gradual removal of urea by dialysis against 10 mM Tris HCl pH 7.5, and examination of the resultant immunoprecipitate by scanning electron microscopy. FIG. 2 shows the typical pentameric structures of HPV6b L1 HEXAHIS protein aggregates.

EXAMPLE 2: Demonstration of antibody production against HPV6b L1 HEXAHIS protein

To produce antibody against HPV6b L1 HEXAHIS protein, mice (strain C57Bl/6) were injected subcutaneously twice at a four week interval with 50 µg protein/mouse following the experimental protocol in Table 2. Two weeks after the second injection the mice were bled.

Serum was obtained from the extracted blood using standard procedures.

The serum was tested for the production of antibodies to HPV6b L1 HEXAHIS protein using three different antigens.

The serum was tested against a human papilloma virus HPV6B capsid preparation. The serum was diluted at 1 in 200 and tested against a HPV6B capsid preparation in RIPA buffer (20 mM Tris-HCl pH 7.6; 2 mM EDTA; 50 mM NaCl; 1% deoxycholate; 1% Triton X-100; 0.25% SDS; 1% aproptinin; and 1 mM PMSF). The antibody-antigen precipitates were run on 10% SDS PAGE separating the individual components of the immune complex. The presence of HPV6b L1 protein was detected with rabbit anti-HPV6b L1 antibody. The presence of HPV6b L1 protein indicates anti-HPV6b L1 antibody was produced in the mouse against 6bL1HEXAHIS protein. Groups A, B, C, D,E and F gave positive results.

Serum was also tested by western blot analysis with HPV6b L1 produced from baculovirus. A positive result indicates anti-HPV6b L1 antibody was produced in the mouse against HPV6b L1 HEXAHIS protein. Groups A, B, C, D, E and F gave positive results with the best result demonstrated when aluminium hydroxide was used as adjuvant. The control groups A, B, C, D and E gave negative results.

The serum was tested by dot blot and ELISA using standard techniques against bovine papilloma virus L1 protein. The best result was achieved with serum from group D mice (i.e. when aluminium was used as an adjuvant) with a OD reading of 0.96. This was followed by serum from group C (i.e. with Freund's complete adjuvant) with OD reading of 0.70, serum from group E (ie. with algammulin) with OD reading 0.34, then serum from group B (i.e. boiled in 1% SDS and cooled) with OD reading 0.24 and serum from group A (no adjuvant) with OD reading 0.34. All control groups had an OD reading of 0.05.

The testing of the serum against three different antigens showed that the HPV6b L1 HEXAHIS protein was immunogenic and produced anti-HPV6b L1 antibodies when used as an antigen with or without adjuvant.

EXAMPLE 3: Demonstration of delayed type hypersensitivity (and confirm antibody production) in mice by HPV6b L1 HEXAHIS protein

Delayed type hypersensitivity involves cell mediated immune reactions as well as some humoral immune reactions. Mice (strain BALB/c) were treated (intraperitoneal injection) with HPV6b L1 HEXAHIS protein under a variety of conditions outlined in Table 3. On day 11 the ear was challenged by intradermal injection) with HPV6b L1 HEXAHIS protein or another HEXAHIS protein. The thickness of the ear was measured on day 13 and day 14. Mice that gave a positive response on day 14 were killed and the histology of the ear was examined.

It was demonstrated in this example that HPV6b L1 HEXAHIS protein without adjuvant induced good delayed type hypersensitivity with initial doses of 50 µg/mouse but not at 5 µg/mouse. However, mice needed to be pertussigen treated to induce a delayed type hypersensitivity response.

With respect to the three examples, it has been shown that HPV6b L1 protein expressed and isolated in the method of Example 1 formed capsomeric aggregates, and the HPV6b L1 protein capsomeric aggregates without further adjuvant were immunogenic producing an antibody response and a cell mediated response. Therefore, HPV6b L1 HEXAHIS protein would serve as a suitable basis for a vaccine designed to prevent human papilloma virus infection by induction of neutralising antibodies or to treat existing lesions through the induction of L1 protein specific cell mediated immunity. Examples 1 to 3 have used HPV6b L1 protein as an example to demonstrate the immunogenicity of the preparation and, as an example, the invention is not restricted to this example and any papilloma virus L1 protein can be used.

EXAMPLE 4: Demonstration that antibodies raised to HPV6b L1 HEXAHIS protein recognise HPV6b L1 virus-like particles (VLPS)

Wells of plates were coated at 0.2 µg protein/well with either HPV6b L1 HEXAHIS produced from *E. coli*, HPV6 VLP-L1 produced from baculovirus, and baculovirus and *E. coli* preparations (cell fermentation

supernatants) as controls, in PBS at pH 7.2 and left to incubate overnight at room temperature. One wash was conducted with PBS at pH 7.2. Non-specific binding was blocked by incubating the plates with 1% (w/v) casein for 1 hour at room temperature.

Rabbit HPV6b L1 HEXAHIS antisera was added to each of the wells coated with HPV6b L1 HEXAHIS, HPV VLP-1, baculovirus prepared controls or *E. coli* prepared controls (prepared in duplicate), and was serially diluted ½ down the plates. Sera raised against influenza virus A/PR-8 was used as a negative control. Sera raised against HPV VLP-L1 was used as positive control on HPV VLP-L1 plates. Plates were incubated for 1 hour, at room temperature, and were then washed three times with PBS containing 0.05% (v/v) Tween 20 at pH 7.2. Goat-rabbit IgG-HRP conjugate was added to each well and plates were incubated and washed as before. Specific binding of antisera to antigen was detected using TMB. The reaction was stopped after 5 minutes using 0.5M HCl.

Results

The results of the experiment are shown in Table 4. Both HPV6b L1 HEXAHIS protein and HPV6 VLP-L1 complexed with antibody raised against HPV6b L1 HEXAHIS protein indicating that HPV6b HEXAHIS L1 correctly presents *in vivo* one or more epitopes presented by HPV VLP-L1. The sera raised against HPV6 L1 protein was also negative in the baculovirus or *E. coli* wells demonstrating the specificity of the reaction. This provides support for the use of HPVL1 HEXAHIS as a vaccine immunogen suitable for inducing antibody which can interact with and potentially neutralise virus. Further, this example provides support for an immunoassay for the detection of papilloma virus L1 protein demonstrated by the coating of various proteins in wells and the use of antibody raised against recombinant HPV6 L1 HEXAHIS protein. Wells containing either HPV6b derived antigen gave a positive result. This example also provides support for an immunoassay for the detection of antibody specific for papilloma virus L1 protein demonstrated by the

coating of the wells with recombinant HPV6b L1 HEXAHIS protein and the use of sera raised against influenza virus A/PR-8 and sera raised against HPV6 L1 HEXAHIS protein. In this case, wells containing sera raised against HPV6 L1 HEXAHIS protein gave a positive result whilst that raised against influenza virus was negative.

EXAMPLE 5: ELISA capture assay demonstrating the formation of multimeric structure-antibody complex

Western blot and ELISA experiments were conducted as previously described or following standard procedures. An ELISA capture assay was conducted by the following method:-

(1) a monoclonal antibody (moAb 8) specific for VLPs was used to coat the wells of a microtitre plate;

(2) HPV VLP L1 protein was added and incubated under suitable conditions and washed with PBS containing 0.1% Tween 20 at pH 7.4;

(3) antibodies raised against various immunogens (shown in column 2 of Table 5) in various animals (shown in column 1 of Table 5) was added; and

(4) suitable detection agents (in the case of rabbit antisera, goat-anti-rabbit peroxidase conjugate was used) were added to detect multimeric structure/VLP-antibody complex.

Results

The amount of captured recombinant papilloma virus HEXAHIS per well is given in Table 5. These experiments demonstrate that the antisera raised against recombinant papilloma virus L1 HEXAHIS proteins elicit an immune response which recognises papilloma virus VLP including L1 protein.

TABLE 1: Procedure for washing the Nickel column with NB buffer

Time (Minutes)	% NB Buffer
0	0
30	0
300	100
310	100
320	100
330	100

TABLE 2: Experimental protocol for injecting mice with 6b L1 HEXAHIS protein to produce antibodies

Mice Group[a]	Addition of 6b L1 HEXAHIS protein[b]	Other conditions
A	+	No adjuvant
A_1	-	No adjuvant
B	+	boiled in 1% SDS and cooled
B_1	-	boiled in 1% SDS and cooled
C	+	with Freund's complete adjuvant
C_1	-	with Freund's complete adjuvant
D	+	absorbed to Aluminium hydroxide
D_1	-	absorbed to Aluminium hydroxide
E	+	with Algammulin
E_1	-	with Algammulin
F	+	with L2 (50 µg) and no adjuvant

TABLE 3: Experimental protocol for producing delayed type hypersensitivity to 6b L1 HEXAHIS protein in mice

Mice Group[a]	Antigen[a]	Adjuvant[c]	Antigen dose	Challenge[b,d]	Pertussigen	Antigen dose	Mean Ear Swelling Day 14[f]
1	L1	PBS	50 µg	L1	+	4	4.5
2	L1	PBS	50 µg	L1	+	13.8	8
3	L1	PBS	50 µg	L1	-	3.8	1.5
4	L1	PBS	50 µg	L1	-	2.4	3.8
5	L1	PBS	50 µg	IRR	+		
6	Saline	PBS		L1	+	4.7	1.3
7	Saline	PBS		L1	-	2.3	1
8	L1	CFA (0.1 ml)	50 µg	L1	+	19.3	16.8
9	Saline	CFA (0.1 ml)		L1	+	4.8	2
10	L1	Quil A (µg)	50 µg	L1	+	15.7	8.7
11	L1	Quil A (µg)	50 µg	L1	-	7.2	0.2
12	L1	Quil A (µg)	50 µg	IRR	+		
13	Saline	Quil A (µg)	50 µg	L1	+		

TABLE 4: Results of ELISA using rabbit HPV6b L1 HEXAHIS antisera

ANTIGEN	ELISA USING RABBIT HPV6b L1 HEXAHIS ANTISERA
HPV6 VLP-L1	> 4.0 exceeds limits @ 1:4000
HPV6b L1 HEXAHIS	2.12 ± 0.1 @ 1:4000
baculovirus control preparation	0.63 ± 0.01 @ 1:4000
E. coli control preparation	0.12 ± 0.00 @ 1:4000

TABLE 5: Results of experiments conducted in Example 5

ANIMAL NO.	IMMUNOGEN	ADJUVANT	IMMUNOREACTIVITY WITH L1 PROTEIN			IMMUNOREACTIVITY WITH L1 ON CELLS	
			Western Blot	ELISA (VLPs)	Capture ELISA	L1 as VLP	L1 as HEXAHIS
Rabbit 31	HEXAHIS L1	CFA	++++	1.712 @ 1:100	0.538 @ 1:100	+++	+++
Rabbit 39	HEXAHIS L1	Nil	++	0.095 @ 1:100	0.050 @ 1:100	+++	?
Rabbit 10	VLPs (baculovirus derived)	Nil	+++	0.972 @ 1:100	0.487 @ 1:100	?	?
Mouse	HEXAHIS L1	Nil	++	?	ND	?	?
Mouse	HEXAHIS L1	CFA	++++	0.400 @ 1:100	ND	?	?
MoAb 8	GST L1 fusion protein	CFA/IFA	++++	++++	ND	+++	?

LEGENDS

TABLE 2

[a] each group of mice contains four mice

[b] 6b L1 HEXAHIS protein was administered at 50 µg protein per mouse.

TABLE 3

[a] Groups consist of 4 to 6 Balb/C mice (68-102)

[b] L1 denotes 6b L1 HEXAHIS protein and IRR denotes irrelevant HEXAHIS protein

[c] PBS is phosphate buffered saline and CFA is complete Freund's adjuvant

[d] 6b L1 HEXAHIS protein was administered at 10 µg in a maximum volume of 2 µl

[e] 30 µg of pertussigen was added

[f] Ear measurements (µm x 10)

TABLE 5

ND: technically cannot be determined

FIGURE 1

DNA nucleotide sequence and amino acid sequence of HPV6b L1 HEXAHIS protein

FIGURE 2

Electron macrograph of pentameric structures of HPV6b L1 HEXAHIS protein aggregates

CLAIMS

1. A recombinant papilloma virus L1 protein which is characterised by its ability to elicit an immune response which recognises papilloma virus VLP including L1 protein and to form extracellularly a multimeric structure, wherein said multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins.

2. A recombinant papilloma virus L1 protein as claimed in Claim 1 wherein the recombinant papilloma virus L1 protein comprises $(His)_6$-papilloma virus L1 protein.

3. A recombinant papilloma virus L1 protein as claimed in Claim 1 or Claim 2 wherein the recombinant papilloma virus L1 protein has the amino acid sequence as shown in FIG. 1.

4. A multimeric structure formed extracellularly and comprising a plurality of recombinant papilloma virus L1 proteins.

5. A multimeric structure as claimed in Claim 4 wherein the multimeric structure is a pentamer.

6. A recombinant DNA molecule encoding a recombinant papilloma virus L1 protein which is characterised by its ability to elicit an immune response which recognises papilloma virus VLP including L1 protein and to form extracellularly a multimeric structure, wherein said multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins.

7. A recombinant DNA molecule as claimed in Claim 6 wherein the recombinant DNA molecule encodes an amino acid sequence as shown in FIG. 1.

8. A recombinant DNA molecule as claimed in Claim 6 or Claim 7 wherein the recombinant DNA molecule has a nucleotide sequence as shown in FIG. 1.

9. A method for preparing a recombinant papilloma virus L1 protein including the steps of:-

(i) expressing a recombinant DNA molecule which encodes the recombinant papilloma virus L1 protein

that is characterised by its ability to elicit an immune response which recognises papilloma virus VLP including L1 protein and to form extracellularly a multimeric structure, wherein said multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins, to form said recombinant papilloma virus protein; and

(ii) purifying said recombinant papilloma virus L1 protein.

10. A method as claimed in Claim 9 wherein the recombinant DNA molecule has a nucleotide sequence as shown in FIG. 1 and the recombinant papilloma virus L1 protein has an amino acid sequence as shown in FIG. 1.

11. A method for detecting the presence of papilloma virus L1 protein in a sample using antibody raised against a recombinant papilloma virus L1 protein which is characterised by its ability to elicit an immune response which recognises papilloma virus VLP including L1 protein and to form extracellularly a multimeric structure, wherein said multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins.

12. A kit for detecting the presence of a papilloma virus L1 protein and including antibody raised against a recombinant papilloma virus L1 protein which is characterised by its ability to elicit an immune response which recognises papilloma virus VLP including L1 protein and to form extracellularly a multimeric structure, wherein said multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins.

13. A method for detecting the presence of antibody specific for papilloma virus L1 protein in a sample using a recombinant papilloma virus L1 protein which is characterised by its ability to elicit an immune response which recognises papilloma virus VLP including L1 protein and to form extracellularly a multimeric structure, wherein said multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins.

14. A kit for detecting the presence of antibody specific for papilloma virus L1 protein in a sample and including a recombinant

papilloma virus L1 protein which is characterised by its ability to elicit an immune response which recognises papilloma virus VLP including L1 protein and to form extracellularly a multimeric structure, wherein said multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins.

15. A vaccine including a recombinant papilloma virus L1 protein which is characterised by its ability to elicit an immune response which recognises papilloma virus VLP including L1 protein and to form extracellularly a multimeric structure, wherein said multimeric structure comprises a plurality of recombinant papilloma virus L1 proteins.

16. A vaccine as claimed in Claim 15 wherein the recombinant papilloma virus L1 protein comprises $(His)_6$-papilloma virus L1 protein.

17. A vaccine as claimed in Claim 15 or Claim 16 wherein the recombinant papilloma virus L1 protein has an amino acid sequence as shown in FIG. 1.

FIG. 1

HEXAHIS leader

ATGCGGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAA
MetArgGlySerHisHisHisHisHisHisGlyMetAlaSerMetThrGlyGlyGlnGln

ATGGGTCGGGATCTGTACGACGATGACGATAAGGAT
MetGlyArgAspLeuTyrAspAspAspAspLysAsp

HPV6BL1

5820 ATGTGGCGGCCTAGCGACAGCACAGTATATGT
TACACCGCCGGATCGCTGTCGTGTCATATACA

MetTrpArgProSerAspSerThrValTyrVal

5880
GCCTCCTCCTAACCCTGTATCCAAAGTTGTTGCCACGGATGCTTATGTTACTCGCACCAA
CGGAGGAGGATTGGGACATAGGTTTCAACAACGGTGCCTACGAATACAATGAGCGTGGTT

ProProProAsnProValSerLysValValAlaThrAspAlaTyrValThrArgThrAsn

5940
CATATTTTATCATGCCAGCAGTTCTAGACTTCTTGCAGTGGGACATCCTTATTTTTCCAT
GTATAAAATAGTACGGTCGTCAAGATCTGAAGAACGTCACCCTGTAGGAATAAAAAGGTA

IlePheTyrHisAlaSerSerSerArgLeuLeuAlaValGlyHisProTyrPheSerIle

6000
AAAACGGGCTAACAAAACTGTTGTGCCAAAGGTGTCAGGATATCAATACAGGGTATTTAA
TTTTGCCCGATTGTTTTGACAACACGGTTTCCACAGTCCTATAGTTATGTCCCATAAATT

LysArgAlaAsnLysThrValValProLysValSerGlyTyrGlnTyrArgValPheLys

6060
GGTGGTGTTACCAGATCCTAACAAATTTGCATTGCCTGACTCGTCTCTTTTCGATCCCAC
CCACCACAATGGTCTAGGATTGTTTAAACGTAACGGACTGAGCAGAGAAAAGCTAGGGTG

ValValLeuProAspProAsnLysPheAlaLeuProAspSerSerLeuPheAspProThr

6120
AACACAACGTTTAGTATGGGCATGCACAGGCCTAGAGGTGGGCAGGGGACAGCCATTAGG
TTGTGTTGCAAATCATACCCGTACGTGTCCGGATCTCCACCCGTCCCCTGTCGGTAATCC

ThrGlnArgLeuValTrpAlaCysThrGlyLeuGluValGlyArgGlyGlnProLeuGly

6180
TGTGGGTGTAAGTGGACATCCTTTCCTAAATAAATATGATGATGTTGAAAATTCAGGGAG
ACACCCACATTCACCTGTAGGAAAGGATTTATTTATACTACTACAACTTTTAAGTCCCTC

ValGlyValSerGlyHisProPheLeuAsnLysTyrAspAspValGluAsnSerGlySer

6240

TGGTGGTAACCCTGGACAGGATAACAGGGTTAATGTAGGTATGGATTATAAACAAACACA
ACCACCATTGGGACCTGTCCTATTGTCCCAATTACATCCATACCTAATATTTGTTTGTGT

GlyGlyAsnProGlyGlnAspAsnArgValAsnValGlyMetAspTyrLysGlnThrGln

6300
ATTATGCATGGTTGGATGTGCCCCCCCTTTGGGCGAGCATTGGGGTAAAGGTAAACAGTG
TAATACGTACCAACCTACACGGGGGGGAAACCCGCTCGTAACCCCATTTCCATTTGTCAC

LeuCysMetValGlyCysAlaProProLeuGlyGluHisTrpGlyLysGlyLysGlnCys

6360
TACTAATACACCTGTACAGGCTGGTGACTGCCCGCCCTTAGAACTTATTACCAGTGTTAT
ATGATTATGTGGACATGTCCGACCACTGACGGGCGGGAATCTTGAATAATGGTCACAATA

ThrAsnThrProValGlnAlaGlyAspCysProProLeuGluLeuIleThrSerValIle

6420
ACAGGATGGCGATATGGTTGACACAGGCTTTGGTGCTATGAATTTTGCTGATTTGCAGAC
TGTCCTACCGCTATACCAACTGTGTCCGAAACCACGATACTTAAAACGACTAAACGTCTG

GlnAspGlyAspMetValAspThrGlyPheGlyAlaMetAsnPheAlaAspLeuGlnThr

6480
CAATAAATCAGATGTTCCTATTGACATATGTGGCACTACATGTAAATATCCAGATTATTT
GTTATTTAGTCTACAAGGATAACTGTATACACCGTGATGTACATTTATAGGTCTAATAAA

AsnLysSerAspValProIleAspIleCysGlyThrThrCysLysTyrProAspTyrLeu

6540
ACAAATGGCTGCAGACCCATATGGTGATAGATTATTTTTTTTTCTACGGAAGGAACAAAT
TGTTTACCGACGTCTGGGTATACCACTATCTAATAAAAAAAAAGATGCCTTCCTTGTTTA

GlnMetAlaAlaAspProTyrGlyAspArgLeuPhePhePheLeuArgLysGluGlnMet

6600
GTTTGCCAGACATTTTTTTAACAGGGCTGGCGAGGTGGGGGAACCTGTGCCTGATACACT
CAAACGGTCTGTAAAAAAATTGTCCCGACCGCTCCACCCCCTTGGACACGGACTATGTGA

PheAlaArgHisPhePheAsnArgAlaGlyGluValGlyGluProValProAspThrLeu

6660
TATAATTAAGGGTAGTGGAAATCGCACGTCTGTAGGGAGTAGTATATATGTTAACACCCC
ATATTAATTCCCATCACCTTTAGCGTGCAGACATCCCTCATCATATATACAATTGTGGGG

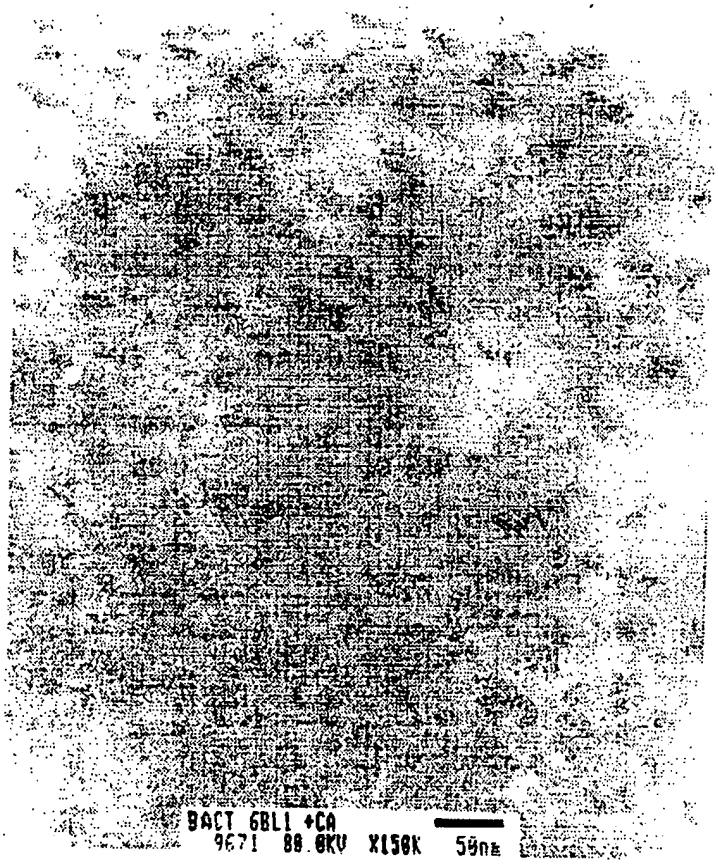
IleIleLysGlySerGlyAsnArgThrSerValGlySerSerIleTyrValAsnThrPro

6720
GAGCGGCTCTTTGGTGTCCTCTGAGGCACAATTGTTTAATAAGCCATATTGGCTACAAAA
CTCGCCGAGAAACCACAGGAGACTCCGTGTTAACAAATTATTCGGTATAACCGATGTTTT

SerGlySerLeuValSerSerGluAlaGlnLeuPheAsnLysProTyrTrpLeuGlnLys

```
6780
AGCCCAGGGACATAACAATGGTATTTGTTGGGGTAATCAACTGTTTGTTACTGTGGTAGA
TCGGGTCCCTGTATTGTTACCATAAACAACCCCATTAGTTGACAAACAATGACACCATCT

AlaGlnGlyHisAsnAsnGlyIleCysTrpGlyAsnGlnLeuPheValThrValValAsp


6840
TACCACACGCAGTACCAACATGACATTATGTGCATCCGTAACTACATCTTCCACATACAC
ATGGTGTGCGTCATGGTTGTACTGTAATACACGTAGGCATTGATGTAGAAGGTGTATGTG

ThrThrArgSerThrAsnMetThrLeuCysAlaSerValThrThrSerSerThrTyrThr


6900
CAATTCTGATTATAAAGAGTACATGCGTCATGTGGAAGAGTATGATTTACAATTTATTTT
GTTAAGACTAATATTTCTCATGTACGCAGTACACCTTCTCATACTAAATGTTAAATAAAA

AsnSerAspTyrLysGluTyrMetArgHisValGluGluTyrAspLeuGlnPheIlePhe


6960
TCAATTATGTAGCATTACATTGTCTGCTGAAGTAATGGCCTATATTCACACAATGAATCC
AGTTAATACATCGTAATGTAACAGACGACTTCATTACCGGATATAAGTGTGTTACTTAGG

GlnLeuCysSerIleThrLeuSerAlaGluValMetAlaTyrIleHisThrMetAsnPro


7020
CTCTGTTTTGGAAGACTGGAACTTTGGGTTATCGCCTCCCCCAAATGGTACATTAGAAGA
GAGACAAAACCTTCTGACCTTGAAACCCAATAGCGGAGGGGGTTTACCATGTAATCTTCT

SerValLeuGluAspTrpAsnPheGlyLeuSerProProProAsnGlyThrLeuGluAsp


7080
TACCTATAGGTATGTGCAGTCACAGGCCATTACCTGTCAAAAGCCCACTCCTGAAAAGGA
ATGGATATCCATACACGTCAGTGTCCGGTAATGGACAGTTTTCGGGTGAGGACTTTTCCT

ThrTyrArgTyrValGlnSerGlnAlaIleThrCysGlnLysProThrProGluLysGlu


7140
AAAGCCAGATCCCTATAAGAACCTTAGTTTTTGGGAGGTTAATTTAAAAGAAAAGTTTTC
TTTCGGTCTAGGGATATTCTTGGAATCAAAAACCCTCCAATTAAATTTTCTTTTCAAAAG

LysProAspProTyrLysAsnLeuSerPheTrpGluValAsnLeuLysGluLysPheSer


7200
TAGTGAATTGGATCAGTATCCTTTGGGACGCAAGTTTTTGTTACAAAGTGGATATAGGGG
ATCACTTAACCTAGTCATAGGAAACCCTGCGTTCAAAAACAATGTTTCACCTATATCCCC

SerGluLeuAspGlnTyrProLeuGlyArgLysPheLeuLeuGlnSerGlyTyrArgGly


7260
ACGGTCCTCTATTCGTACAGGTGTTAAGCGCCCTGCTGTTTCCAAAGCCTCTGCTGCCCC
TGCCAGGAGATAAGCATGTCCACAATTCGCGGGACGACAAAGGTTTCGGAGACGACGGGG

ArgSerSerIleArgThrGlyValLysArgProAlaValSerLysAlaSerAlaAlaPro

          TAAACGTAAGCGCGCCAAAACTAAAAGGTAA  7291
          ATTTGCATTCGCGCGGTTTTGATTTTCCATT

          LysArgLysArgAlaLysThrLysArgTer
```

FIG. 2



INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 95/00292

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C07K 14/025, 16/08, C12N 15/37, A61K 39/12, G01N 33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: FILE WPAT as below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU:FILE WPAT as below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
FILE WPAT(Derwent Database): Keywords: Papillomavirus, Papilloma Virus
FILE CASM: Papillomavirus, Papilloma virus, Late, L1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	AU 64436/94 A (UNIVERSITY OF ROCHESTER) 26 September 1994, see especially page 5 lines 23 to 30; example I page 19 lines 1 to 15.	1 to 17
X,Y	WO 94/05792 A (THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by the SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 17 March 1994, see especially page 4 line 26 to page 5 line 2.	1 to 17
X	WO 94/00152 A (GEORGETOWN UNIVERSITY) 6 January 1994, see especially page 8 lines 6 to 8; example 2 pages 37 to 40.	1 to 17

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
	18 July 1995 (18.07.95)
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 2853929	Authorised officer M ROSS Telephone No.: (06) 2832295

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AU 23666/92 A (THE UNIVERSITY OF QUEENSLAND et al) 23 February 1993, see whole document.	1 to 17
P,X	Sasagawa et al, Virology, Volume 206 (1), (1995) pages 126 to 135. "Synthesis and Assembly of Virus-like Particles of Human Papillomaviruses Type 6 and Type 16 in Fission Yeast Schizosaccharomyces pombe" see especially page 131, column 1, lines 14 to 18; Figure 6 page 132.	1 to 17
X,Y	Cason et al. Biochemical Society Transactions, Volume 22 (3) (1994) page 3355 "Detection of protein aggregrates, but not virus-like particles, when the major (L1) coat protein of a wild-type human papillomavirus type 16 (HPV-16) is expressed in insect cells" see whole document.	1 to 17
P,X	Chemical Abstracts, Volume 121 (7) (15 August 1994) page 572, "Self-assembly of human papillomavirus type 16 capsids by expression of the L1 protein in insect cells", column 2, abstract no. 78067, Le Cann et al. FEMS Microbiol. Lett. (1994) 117 (3), 269-74.	1 to 17
X	Kirnbauer et al. Journal of Virology, Volume 67 (12) (1993) pages 6929 to 6936. "Efficient Self-Assembly of Human Papillomavirus Type 16 L1 and L1-L2 into Virus-Like Particles" see whole document.	1 to 17
X	Rose et al. Journal of Virology, Volume 67(4) (1993) pages 1936 to 1944. "Expression of Human Papillomavirus Type 11 L1 Protein in Insect Cells: In Vivo and In Vitro Assembly of Virus-like Particles". see whole document.	1 to 17
X	Hagensee et al. Journal of Virology, Volume 67(1) (1993) pages 315 to 322. "Self-Assembly of Human Papillomavirus Type 1 Capsids by Expression of the L1 Protein Alone or by Coexpression of the L1 and L2 Capsid Proteins". see especially page 315 column 2 line 1 to 3; Figure 6 page 320.	1 to 17
Y	Zhou et al. Journal of General Virology, Volume 74 (1993) pages 763 to 768. "Synthesis and assembly of infectious bovine papillomavirus particles in vitro". see whole document.	1 to 17
X	Kirnbauer et al. Proc. Natl. Acad. Sci, USA, Volume 89 (1992) pages 12180 to 12184. "Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic" see especially page 12180 column 2 lines 2 to 7; page 12182 column 1 line 21 to column 2 line 8.	1 to 17
Y	Xi et al. Journal of General Virology, Volume 72 (1991) pages 2981 to 2988. "Baculovirus expression of the human papillomavirus type 16 capsid proteins: detection of L1-L2 protein complexes". see whole document.	1 to 17
X	Chemical Abstracts, Volume 119 (21) (22 November 1993) page 783, "Papillomavirus L1 major capsid protein self-assembly into particles that are morphologically and immunologically similar to native virons", column 2, abstract no. 223679. Kirnbauer et al. Vaccines 95 [Annu. Meet.], 10 1992 (Pub 1993) 379-83.	1 to 17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/AU 95/00292

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
AU	64436/94	WO	9420137		
WO	9400152	EP	647140		
AU	23666/92	EP	595935	WO	9302184

END OF ANNEX